

What is claimed is:

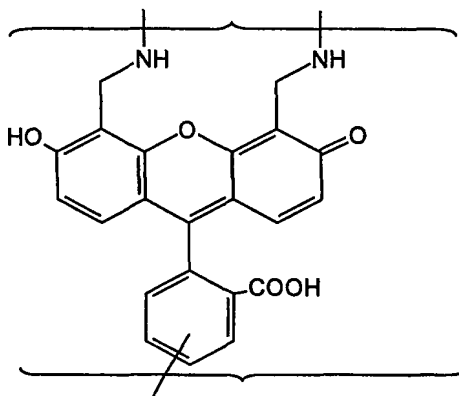
1. A compound comprising:

i) a first fluorochrome having first absorption and emission spectra;

and

ii) at least one of a second fluorochrome each said second fluorochrome being covalently attached through a linker group to said first fluorochrome and each second fluorochrome having second absorption and emission spectra, the wavelength of the emission maximum of the second fluorochrome(s) being longer than the emission maximum of the first fluorochrome and a portion of the absorption spectrum of each of said second fluorochromes overlapping a portion of the emission spectrum of said first fluorochrome such that each of said second fluorochromes is capable of accepting energy from said first fluorochrome; and

wherein said first fluorochrome comprises a radical of the dye 4', 5'-bis-aminomethylfluorescein having the formula:



2. The compound according to claim 1 wherein said compound includes at least one target bonding group capable of forming a covalent bond with a target material.

3. The compound according to claim 1 or 2 further comprising:

charge carrying substituents or water solubilizing substituents,
covalently attached thereto, or

charge carrying and water solubilizing substituents, covalently
attached thereto,

5 said substituents being unreactive with said target bonding group.

4. The compound according to claim 3 wherein said water solubilizing
substituents are selected from the group consisting of amide, sulphonate,
sulphate, phosphate, quaternary ammonium, hydroxyl, guanidinium and
10 phosphonate.
5. The compound according to claim 3 wherein said charge carrying
substituents incorporate from one to five positively charged nitrogen or
phosphorus atoms.
- 15 6. The compound according to claim 2 wherein said target bonding group is a
reactive group selected from the group consisting of N-
hydroxysuccinimidyl ester, N-hydroxy-sulphosuccinimidyl ester,
isothiocyanate, haloacetamide, dichlorotriazine, maleimide, sulphonyl
20 halide, acyl halide, anhydride and phosphoramidite.
7. The compound according to claim 2 wherein said target bonding group is a
functional group selected from the group consisting of amino, hydroxyl,
sulphydryl, and carboxyl groups.
- 25 8. The compound according to claim 1 wherein each of said second
fluorochromes are selected from xanthine dyes, rhodamine dyes and
cyanine dyes.
- 30 9. The compound according to claim 2 wherein said target material is
selected from the group consisting of: antibodies, lipids, proteins, peptides,
carbohydrates nucleotides containing or are derivatized to contain one or
more amino, sulphydryl, carbonyl, hydroxyl, carboxyl, phosphate, or

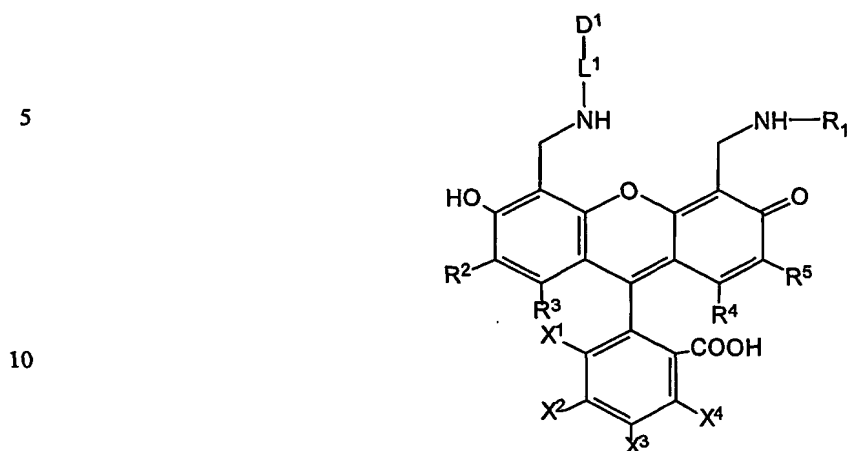
thiophosphate groups; oxy or deoxy polynucleic acids containing or are derivatized to contain one or more amino, sulphydryl, carbonyl, hydroxyl, carboxyl, phosphate, or thiophosphate groups; microbial materials, drugs, hormones, cells, cell membranes and toxins.

5

10. The compound according to claim 1 having a plurality of said second fluorochromes each covalently attached through a linker to said first fluorochrome, and each of said second fluorochromes being capable of accepting energy from said first fluorochrome when said first fluorochrome is excited by light.
- 10
11. The compound according to claim 1 further comprising two or more first fluorochromes linked in an energy transfer relationship with a second fluorochrome and wherein each said first fluorochrome comprises a radical of the dye 4', 5'-bis-aminomethylfluorescein-5(6)-carboxylic acid and said two or more first fluorochromes being covalently linked head to tail through the 4'- (or 5'-) amino and carboxyl groups of said radical.
- 15
12. The compound according to claim 1 further comprising one or more third fluorochromes covalently attached to said first or second fluorochromes, and each third fluorochrome having third absorption and emission spectra, the wavelength of the emission maximum of said third fluorochrome(s) being longer than the wavelength of the emission maximum of said second fluorochrome and a portion of the absorption spectrum of each of said third fluorochrome(s) overlapping a portion of the emission spectrum of said second fluorochrome such that excitation of said first fluorochrome produces fluorescence from said third fluorochrome(s).
- 20
- 25

30

13. A compound having the structure:



wherein:

15 D¹ is an acceptor dye selected from the group consisting of xanthine dyes, rhodamine dyes and cyanine dyes;

R¹ is selected from H, an amino-protecting group, the group -L²- F and the group -L²- D², where D² is a dye selected from the group consisting of xanthine dyes, rhodamine dyes and cyanine dyes;

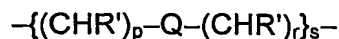
20 R², R³, R⁴ and R⁵ independently represent H, F, Cl, C₁ - C₆ alkyl, C₁ - C₆ substituted alkyl, C₁ - C₆ alkoxy, sulfonate, sulfone, amido, nitrile, aryl or heteroaryl; or R² and R³ and/or R⁴ and R⁵ taken together may be linked to form a fused aromatic or heteroaromatic ring system;

25 X¹, X², X³ and X⁴ independently represent H, F, Cl, C₁ - C₆ alkyl, C₁ - C₆ alkenyl, C₁ - C₆ alkynyl, COOR', SO₃H, CH₂OH, the group -L³- F and the group -L³- D³, where D³ is a dye selected from the group consisting of xanthine dyes, rhodamine dyes and cyanine dyes; and R' is selected from hydrogen and C₁ - C₄ alkyl;

F is a target bonding group; and

30 L¹, L² and L³ are each a linking group and each independently comprises a group containing from 1 to 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from -C(O)-, -C(S)-, -NR'-, -O-, -S-, -CR'=CR'- and -CO-NR'- groups, where R' is hereinbefore defined.

14. The compound according to claim 13 wherein said compound includes at least one target bonding group capable of forming a covalent bond with a target material.
- 5 15. The compound according to claim 13 or 14 further comprising:
charge carrying or water solubilizing substituents covalently attached thereto, or
charge carrying and water solubilizing substituents covalently
10 attached thereto,
said substituents being unreactive with said target bonding group.
16. The compound according to claim 15 wherein said water solubilizing substituents are selected from the group consisting of amide, sulphonate,
15 sulphate, phosphate, quaternary ammonium, hydroxyl, guanidinium and phosphonate.
17. The compound according to claim 15 wherein said charge carrying substituents incorporate from two to five positively charged nitrogen
20 atoms.
18. The compound according to claim 13 wherein each of L^1 , L^2 and L^3 independently contains from 1 to 20 atoms.
- 25 19. The compound according to claim 13 wherein L^1 , L^2 and L^3 are each independently:



- 30 where Q is selected from: $-\text{CHR}'-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{NR}'-$, $-\text{O}-$, $-\text{CR}'=\text{CR}'-$ and $-\text{CO}-\text{NR}'-$; R' is hydrogen or $\text{C}_1 - \text{C}_4$ alkyl, each p is independently 0 – 5, each r is independently 0 – 5 and s is 1 or 2.

20. The compound according to claim 19 wherein Q is selected from $-\text{CHR}'-$, $-\text{C}(\text{O})-$ and $-\text{CO}-\text{NH}-$, where R' , p, r and s are hereinbefore defined.
21. The compound according to claim 14 wherein said target bonding group
5 comprises a reactive group for reacting with a functional group on a target material, or a functional group for reacting with a reactive group on a target material.
22. The compound according to claim 21 wherein said reactive group is
10 selected from the group consisting of N-hydroxysuccinimidyl ester, N-hydroxy-sulphosuccinimidyl ester, isothiocyanate, haloacetamide, dichlorotriazine, maleimide, sulphonyl halide, acyl halide, anhydride and phosphoramidite.
23. The compound according to claim 21 wherein said functional group is
15 selected from the group consisting of amino, hydroxyl, sulphydryl, and carboxyl groups.
24. The compound according to claim 13 wherein said xanthine dye is
20 selected from fluorescein, naphthofluorescein, rhodol and derivatives thereof.
25. The compound according to claim 13 wherein said rhodamine dye is
25 selected from 5-carboxyrhodamine (Rhodamine 110-5), 6-carboxyrhodamine (Rhodamine 110-6), 5-carboxyrhodamine-6G (R6G-5 or REG-5), 6-carboxyrhodamine-6G (R6G-6 or REG-6), N,N,N',N'-tetramethyl-5-carboxyrhodamine, N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA or TMR), 5-carboxy-X-rhodamine, 6-carboxy-X-rhodamine (ROX).
- 30 26. The compound according to claim 13 wherein said cyanine dye is selected from Cy3 (3-(ϵ -carboxypentyl)-1'-ethyl-3, 3, 3', 3'-tetramethyl-5, 5'-disulphonato-carbocyanine), Cy3.5 (3-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-

tetramethyl-4,5,4',5'-(1,3-disulphonato)dibenzo-carbocyanine), Cy5 (1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethyl-5,5'-disulphonato-dicarbocyanine, Cy5.5 (1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethyl-4,5,4',5'-(1,3-disulphonato)-dibenzo-dicarbocyanine, Cy7 (1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethyl-5,5'-disulphonato-tricarbocyanine.

27. The compound according to claim 14 wherein said target material is selected from the group consisting of: antibodies, lipids, proteins, peptides, carbohydrates, nucleotides containing or are derivatized to contain one or more amino, sulphydryl, carbonyl, hydroxyl, carboxyl, phosphate or thiophosphate groups; oxy or deoxy polynucleic acids containing or are derivatized to contain one or more of an amino, sulphydryl, carbonyl, hydroxyl, carboxyl, phosphate or thiophosphate groups; microbial materials, drugs, hormones, cells, cell membranes and toxins.
28. A method for labelling a target material comprising:
- a) adding to a liquid containing said target material a fluorescent energy transfer reagent according to claim 1 or claim 13; and
 - b) incubating said reagent with said target material under conditions suitable for binding to and thereby labelling said target material.
29. The method according to claim 28 wherein said target material is selected from the group consisting of: antibodies, lipids, proteins, peptides, carbohydrates, nucleotides containing or are derivatized to contain one or more amino, sulphydryl, carbonyl, hydroxyl, carboxyl, phosphate or thiophosphate groups; oxy or deoxy polynucleic acids containing or are derivatized to contain one or more amino, sulphydryl, carbonyl, hydroxyl, carboxyl, phosphate or thiophosphate groups; microbial materials, drugs, hormones, cells, cell membranes and toxins.